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CARDIOVASCULAR GENOMIC MEDICINE

Cardiovascular Proteomics

Tools to Develop Novel Biomarkers and Potential Applications

Sara Arab, PHD,*¶ Anthony O. Gramolini, PHD,*† Peipei Ping, PHD,# Thomas Kislinger, PHD,‡§ Brian Stanley, PHD,‖** Jennifer van Eyk, PHD,‖** Maral Ouzounian, MD,*‖ David H. MacLennan, PHD,*‡ Andrew Emili, PHD,*‡ Peter P. Liu, MD*¶

Toronto, Ontario, Canada; Los Angeles, California; and Baltimore, Maryland

Proteomics is the new systems biological approach to the study of proteins and protein variation on a large scale as a result of biological processes and perturbations. The field is undergoing a dramatic transformation, owing to the completion and annotation of the human genome as well as technological advances to study proteins on a large scale. The new science of proteomics can potentially yield novel biomarkers reflecting cardiovascular disease, establish earlier detection strategies, and monitor responses to therapy. Technological advances permit the unprecedented large-scale identification of peptide sequences in a biological sample with mass spectrometry, whereas gel-based techniques provide further refinement on the status of post-translational modification. The application of high throughput protein evaluation with a subset of predefined targets, identified through proteomics, microarray profiling, and pathway analysis in animal models and human tissues, is gaining momentum in research and clinical applications. Proteomic analysis has provided important insights into ischemic heart disease, heart failure, and cardiovascular pathophysiology. The combination of proteomic biomarkers with clinical phenotypes and genetic haplotype information can lead to a more precise diagnosis and therapy on an individual basis—the fundamental premise of "personalized medicine." (J Am Coll Cardiol 2006;48:1733–41)
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The social, economic, and human costs of cardiovascular disease continue to escalate. Despite the amazing scientific advances in the physical and biological sciences, the number of effective cardiovascular therapies and viable therapeutic targets remains surprisingly limited. The number of useful cardiovascular biomarkers is even fewer.

To gain an insight into the disease processes in individual patients, their unique response to etiological risk factors, and their stages of disease progression, an array of diagnostic tools such as haplotype analysis for genes of susceptibility, gene expression arrays (transcriptome), metabolic profiling (metabolome), and new protein profiling tools (proteome) are moving from the bench to the bedside. These technologies, sometimes referred to as the "new science," might provide the opportunity to facilitate the transition from the current paradigms of generic clinical decision-making to the new era of a more "personalized medicine."

From the *Heart and Stroke/Richard Lewar Center of Excellence, University of Toronto and the Canadian Institutes of Health Research, Ottawa, Ontario, Canada; †Department of Physiology, ‡Banting and Best Department of Medical Research and §Program in Proteomics and Bioinformatics, University of Toronto, Toronto, Ontario, Canada; ‖Queen's University, Kingston, Ontario, Canada; ¶Department of Medicine, University of Toronto, Toronto, Ontario, Canada; #University of California Los Angeles–David Geffen School of Medicine, Los Angeles, California; and **Johns Hopkins NHLBI Proteomics Center, Baltimore, Maryland. Drs. MacLennan, Gramolini, Emili, and Liu receive a partnership research grant through Genome Canada and Roche Diagnostics GmbH; Dr. van Eyk is scientific director of Cardiomics, Inc. Cardiovascular Genomic Medicine series edited by Geoffrey S. Ginsburg, MD, PhD.

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"Proteomics" was coined by Australian researchers a decade ago to describe all the proteins expressed by the genome that defines an organism. Proteomics is thus the study of the large-scale expression, function, and interaction of the complement of proteins in an organism in health and disease. Recent advances in proteomic technologies permit the evaluation of systematic changes in protein expression in response to intrinsic or extrinsic perturbations to the biologic system, for example, those that occur in cardiovascular disease. Proteomics is a potential tool for the discovery and application of novel biomarkers in diagnosis of the inception and progression of heart disease, which might then affect prevention and therapy.

Proteomics differs from "genomics" in both complexity and dynamic variability. Whereas the genome is relatively constant, the proteome is constantly changing according to the moment-to-moment interactions between the genome and the environment. It differs from "metabolomics," or metabolic profiling, because the latter details the metabolic intermediates as a result of protein enzymatic actions on the energetic substrates. Proteomics differs from traditional "protein chemistry" in that the latter studies mainly the chemical properties of specific families of proteins, whereas proteomics studies the large-scale interaction of the protein repertoire within an organism. Proteomic biomarkers also differ from traditional biochemical markers that we currently use clinically, in that multiple interacting protein species are evaluated simultaneously to reflect the re-

Abbreviations and Acronyms
ELISA = enzyme-linked immunoadsorbent assay
HUPO = Human Proteomic Organisation
PTM = post-translational modification

sponse of a cell or an organism to disease or adversities in the environment.

Proteomic analysis provides a unique opportunity to understand the pathophysiology of disease in a non-biased manner. It also permits the development of a suite of candidate biomarkers for the diagnosis, staging, and tracking of disease. Yet no technology, however sophisticated, can provide scientific insights and advances without the accompaniment of proper clinical information and the biological context.

APPLICATIONS OF PROTEOMICS IN CARDIOVASCULAR DISEASE

Understanding of novel pathophysiological mechanisms. One major benefit afforded by current large-scale proteomic technologies, regardless of method or combination of methods used, is the potential for a greater understanding of the cellular processes that are responsible for the transition to disease phenotypes. By profiling the changes in the total detectable protein pool of the heart in a time- and disease-dependent manner, it is potentially possible to evaluate the changes in critical pathways that might be present early in the disease process, before the onset of clinical symptoms or functional perturbations and during disease progression. The knowledge might also help to distinguish etiological background, individualized pathophysiology, and in turn, prognosis and individualized therapy. The new proteomic tools, with systems biology approaches, have the potential to unravel greater complexity in previously unexplored signaling networks, leading to improved biological understanding and evaluation as potential novel biomarkers.

In addition to quantification of protein levels, large-scale proteomic studies have the ability to detect post-translational modifications (PTMs). Although cataloguing PTMs, such as phosphorylation, sulphation, glycosylation, hydroxylation, nitrosylation, N-methylation, carboxymethylation, acetylation, prenylation, and N-myristoylation, is still early in development, progress has been rapid and shows promise.

Identification of diagnostic/prognostic biomarkers for cardiovascular disease. Cardiovascular diseases traditionally have relied on epidemiological associations to identify diagnostic and prognostic risk factors, such as hypercholesterolemia and hypertension in populations. Only a handful of biomarkers such as troponin for acute coronary syndromes or brain natriuretic peptide for acute heart failure are currently used to diagnose or prognosticate individual patients.

With advances in proteomic technologies, the approach of biomarker identification now shifts from large-scale epidemiologic associations to association between the disease phenotype and specific genomic or proteomic patterns. These novel biomarkers can be developed for increased precision in diagnosis, identification of susceptibility to complications, and prognosis tailored to individuals and subclassification of disease on the basis of pathophysiological manifestations. Because individual biomarkers will have limited sensitivity and specificity, proteomics affords the opportunity to identify a panel of complementary biomarkers that will have more robust operational characteristics.

Whereas a good diagnostic marker might or might not have known biological relevance to the disease process (e.g., C-reactive protein might behave as a good risk factor by association without having been proven to have a causative role in disease formation), we believe that most useful or specific diagnostic/prognostic/efficacy biomarkers eventually will be shown to have biological relevance to the disease condition. Therefore, in our search for novel biomarkers for heart failure or atherosclerosis, we look for convergence of proteomic patterns observed from both relevant animal models of the disease and from robust clinical phenotypes showing the same disease. Potential biomarkers can be identified with timed evaluation of animal models of disease development. The subsequent validation of these candidate suites of biomarkers will be most critical to distinguish those that will be robust for clinical application versus those that do not provide adequate sensitivity or specificity or biological insight into disease progression.

Identification of novel therapeutic targets. The combination of animal model and/or clinical studies will naturally lead to novel insights into the biology of the disease under study. Additional in-depth evaluation of new biomarker pathways will identify a number of potential novel targets relevant to the disease. Depending on the nature of these targets and ease with which the disease can be manipulated therapeutically, proteomic tools allow for screening of targets, their ligands and interacting partners, and even therapeutic candidates and predictors of response. Overall, medical diagnostics will most likely be the first goals to be achieved from proteomic technologies. It will take longer to evaluate therapeutic target function and identify safe and effective modulators that are suitable for clinical development.

EXAMPLES OF PROTEOMIC BIOMARKER CANDIDATES AND IMPLICATIONS FOR APPLICATION

Emerging proteomic biomarkers as applied to ischemic heart disease. The evaluation of tissues in models of myocardial ischemia has confirmed traditional protein markers of ischemia and tissue defense and injury. In a rabbit model of ischemia reperfusion, 2-dimensional (2-D) gel analysis revealed systematic perturbations of several classes of known and novel proteins. These include sarcomere and cytoskeleton proteins (myosin light chain and troponin C); redox enzymes (reduced nicotinamide adenine dinucleotide [NADH] ubiquinon oxidoreductase com-

plexes); energy metabolism (including creatine kinase isoforms); and stress response proteins (1). More sustained ischemia in a pig model also revealed systematic alterations in other families of proteins. These included elevations of cathepsin B and D, heat shock protein Hsc73, and beclin1, all associated with autophagy of tissues (2). These protein changes are accompanied by a decrease in apoptosis, suggesting that these proteins are activated to protect the myocardial tissue against ischemia.

Plasma sample analysis of patients with acute coronary syndromes has also identified interesting associated biomarkers to date. Two-dimensional gel electrophoresis analysis of plasma from patients with myocardial infarction compared with unaffected individuals showed a reduction of families of alpha(1)-antitrypsin, fibrinogen, and apolipoprotein A-I isoforms in myocardial infarction or unstable angina (3). In contrast, gamma-immunoglobulin heavy chains were increased in acute coronary syndrome. Even for the traditional troponin I and T used for current diagnosis of myocardial injury, Labugger et al. (4) have identified multiple post-translationally modified, truncated, or degraded protein forms that are reflective of the underlying degree of injury.

Emerging proteomic insights in heart failure. Analysis of ventricular proteomic changes during the initial inception, development, and progression to heart failure revealed large-scale pattern differences (5). Major alterations observed in heart failure relate to inflammation, calcium signaling, growth and deaths, and cytoskeletal/matrix remodeling targets. In more specific genetic models of cardiomyopathy, such as Rac1 transgenic mice, proteins regulated in heart failure include creatine kinase M-chain, tubulin beta-chain, manganese superoxide dismutase, and malate dehydrogenase (6). Similarly, in canine models of acute heart failure due to ischemia, certain proteins are up-regulated, including nicotinamide adenine dinucleotide (NAD+) isocitrate dehydrogenase and mitochondrial adenosine triphosphate (ATP) synthase D chain, whereas creatine kinase M chain and myosin light chain-1 were decreased (7).

In terms of plasma, the aforementioned protein candidates are also consistent with the efforts of the Human Proteomic Organisation (HUPO) to analyze the human plasma subproteome systematically. Initial analysis identified families of proteins involved in inflammation, signaling, growth and differentiation, cytoskeletal, channel/receptors, and remodeling processes (8).

PROTEOMIC TECHNOLOGICAL PLATFORMS

Gel-based proteomic approaches. In cardiovascular proteomics, much of the work to date has been performed through high-quality 2-D gel-based electrophoresis of tissue or blood samples (9–11). The principles of the gel-based technique involve separation of proteins in the first dimension according to their charge properties (isoelectric point [pI]) under denaturing conditions, followed by their separation in the second dimension according to their relative molecular mass by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) (12–14).

Studies using gel-based technologies have yielded examples of novel insights into the mechanisms of ischemic myocardial disease, particularly the *post-translational modifications* of abundant proteins, such as troponin (10). Gel-based techniques have the additional advantages of ease of use and cost efficiency. However, the technique is constrained in terms of depth of protein coverage. Given that any biological protein sample displays substantial complexity and can span protein expression levels of up to 10 orders of magnitude, gel-based proteomic techniques tend to bias toward detection of high-abundance housekeeping enzymes, with reduced detection of low-abundance membrane-bound proteins or those with extremes in isoelectric point (15,16). However, recent advances in selective expansion of narrow pH ranges have increased significantly the resolution of this technique (10) (Fig. 1). The ability to deplete dominating protein species such as albumin in the blood and contractile and structural proteins in the myocardium has also facilitated the detection of smaller molecular weight species important in regulation and signaling. In the current era, gel-based techniques are particularly useful for specific target validation when the protein species are known, for example, through identification by mass spectrometry. They are also suitable for the evaluation of PTM.

Isotope affinity tagged techniques. A complementary approach to the gel-based proteomic technique is the use of isotope affinity tags to increase the sensitivity of detection of smaller peptide fragments. This can be done with or without prior protein or organelle separation. The first breakthrough in quantifying differences in protein composition between 2 samples was the development of isotope coded affinity tags (ICAT), which selectively label cysteine residues of peptide fragments following tryptic digest of the protein sample. The labeled fraction is then selectively analyzed and has been found to increase significantly the depth of protein coverage but is limited by selective labeling. A more recent improvement led to the iTRAQ technique (Applied Biosystems, Foster City, California) in which every tryptic fragment is labeled, thus increasing the confidence in the protein species identified. The iTRAQ technique also allows for measurement of absolute rather than relative changes in protein composition, thereby permitting comparisons across multiple samples.

Gel-free "shotgun" proteomic techniques with tandem mass spectrometry. The more recent use of gel-free systems, which couple high-efficiency liquid chromatography separation procedures with automated tandem mass spectrometry, allows for large-scale "shotgun" sequencing of complex mixtures (5,17,18). The archetypal approach, termed MudPIT (for multidimensional protein identification technology) (19), has proven to be effective for investigating global changes in protein expression as a function of development and disease

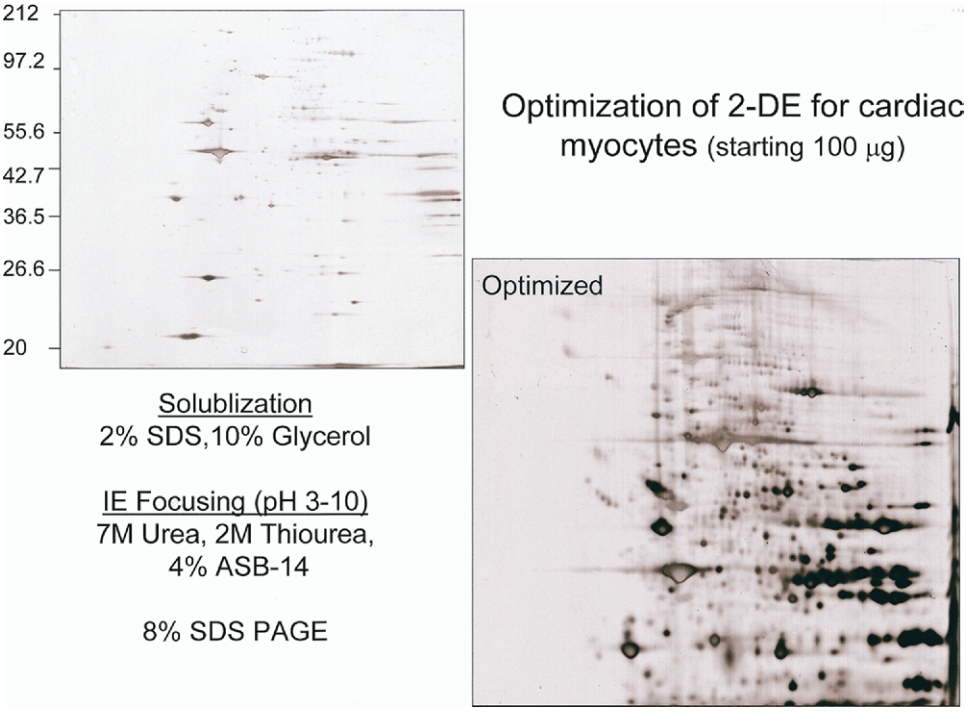

Figure 1. Large-scale protein analysis of myocytes can be done with 2-dimensional gel electrophoresis (2-DE), where the proteins can be separated and identified on the basis of molecular weight and pH. However, to improve the resolution and to achieve greater protein separation, optimization of the gel processing conditions (e.g., expanding the pH range by selective isoelectric [IE] focusing) helps to improve the resolution significantly (for details, see Stanley et al. [37]). ASB = amidosulfobetaine; SDS PAGE = sodium dodecylsulfate polyacrylamide gel electrophoresis.

(Fig. 2) (20–22). The advantages in terms of scale and efficacy are, however, balanced by a more complex algorithm and less direct means to gain insight into PTM or isoform shifts.

In contrast to 2-D electrophoretic techniques in which high-abundance protein species predominate, the gel-free "shotgun" profiling procedures provide much more extensive coverage with orders of magnitude increase in resolution. The tandem approach permits peptide species identification and also semi-quantitative estimation of the relative abundance. Current efforts are focused on adapting data mining algorithms, which can be trained to find specific features to distinguish between or classify new samples. Shotgun techniques require typically rigorous statistical approaches to evaluate the significance of any predicted patterns, owing to the volume of data analyzed and the hazards of multiple comparisons.

Shotgun tandem mass spectrometry technique currently provides the greatest depth of protein coverage and widest dynamic range of size of proteins identified. Once the candidates are identified, validation can be done with gel-based approaches or dedicated protein arrays.

Protein arrays in targeted sample analysis. In contrast to the system biology approaches in which global proteomic

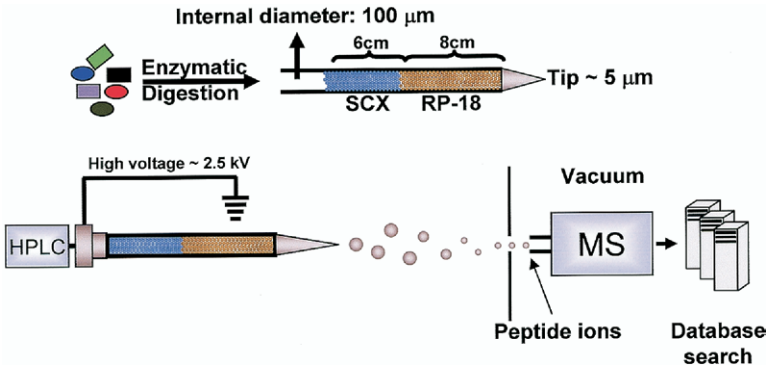

Figure 2. "Shotgun" proteomic analysis combining liquid chromatography with mass spectrometry (LC-MS) or so-called multidimensional protein identification technology (MudPIT). In this approach, proteins are enzymatically digested and subjected to fractionation through cation exchange and reverse phase, and the peptide fragments are sprayed through high voltage into the mass spectrometer. The unique emerging spectra are then recorded and compared with existing databases to search for their identity. HPLC = high-pressure liquid chromatography; MS = mass spectometry; RP = reverse phase; SCX = strong cation exchange.

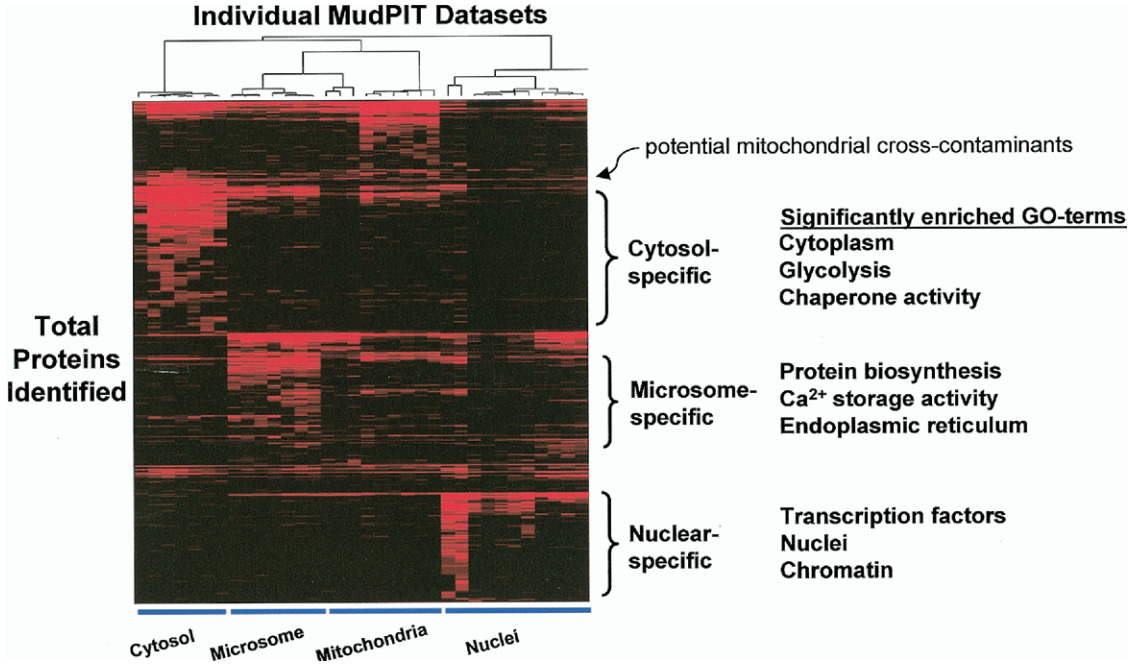

Figure 3. Cluster analysis of mouse myocardial peptide fractions emerging from "shotgun" processing. The peptides naturally cluster into cytosolic, microsomal, mitochondrial, and nuclear fractions. The peptide localization confers functional specificity, such that the calcium regulatory proteins are much more abundant in the microsomal fraction, whereas transcription factors are in the nuclear-specific fractions. Ca^{2+} = calcium; GO = Gene Ontology; MudPIT = multidimensional protein identification technology.

patterns are determined, it is now possible to interrogate a sample with a panel of a specified set of protein targets with customized protein chips or arrays. Different platforms of protein microarrays are now readily accessible. A popular platform is the forward phase protein microarrays or antibody arrays, which consist of a series of immobilized antibodies on a membrane or solid media to provide relative protein expression levels for a specific sample (23,24). This method is particularly useful in evaluating a large number of similar samples in a high throughput manner, for a small number of specific protein targets. However, different antibody affinities and microreaction kinetics might lead to false readings when the entire array is processed under identical conditions.

The recent development of reverse phase protein microarrays provides an alternative to classic antibody arrays. The reverse phase protein microarrays are fabricated by depositing small volumes of a large number of protein samples or cell lysates onto a high protein-binding substratum with a robotic precision spotter. Each protein microspot contains the full complement of the representative sample, including phosphorylated analytes, as appropriate. Because thousands of samples can be spotted in high density onto a single slide, a large number of samples can be monitored simultaneously (25). However, because the same antibody is being used, the comparisons between samples will be more valid (26). Protein microarrays could be used to screen binding of specific inhibitors or ligands to receptors, evaluation of enzymatic activity, protein–protein, protein–lipid, and protein–DNA interaction profiling in answering a variety of biological questions. Interestingly, autoantibodies can also be detected with reverse arrays, which is particularly useful for the investigation of autoimmune related disorders such as cardiomyopathies.

Classical enzyme-linked immunosorbent assays. To quantify specific proteins in a large number of samples with high sensitivity and precision, the classic enzyme-linked immunoadsorbent assay (ELISA) or its variation is still the reliable standard. The ELISA immunoassay requires the availability of a specific primary antibody to the antigen of interest. The antibody-antigen complexes are then captured onto a solid surface, and the antibody is in turn conjugated to an enzyme capable of generating a colored substrate for light detection and quantification against a standard.

The ELISAs are extremely sensitive, capable of detecting proteins in the picomolar to nanomolar ranges (10^{-12} to 10^{-9} mol/l), with short assay time and high throughput. However, ELISA by definition requires the prior availability of a high affinity specific antibody. Therefore, ELISAs are excellent tools to complement the discovery platforms discussed previously. To evaluate biomarkers in large samples of clinical materials, the development of appropriate ELISAs for the protein targets with high affinity antibodies is an essential part of the program.

PROTEOMIC PROCESSING CONSIDERATIONS

Importance of considering subproteomes. The ability to differentiate individual species of peptides by any method is dependent on the number of peptides in the sample. To analyze any protein sample in depth and maximize the yield, it is important to consider fractionating the sample into

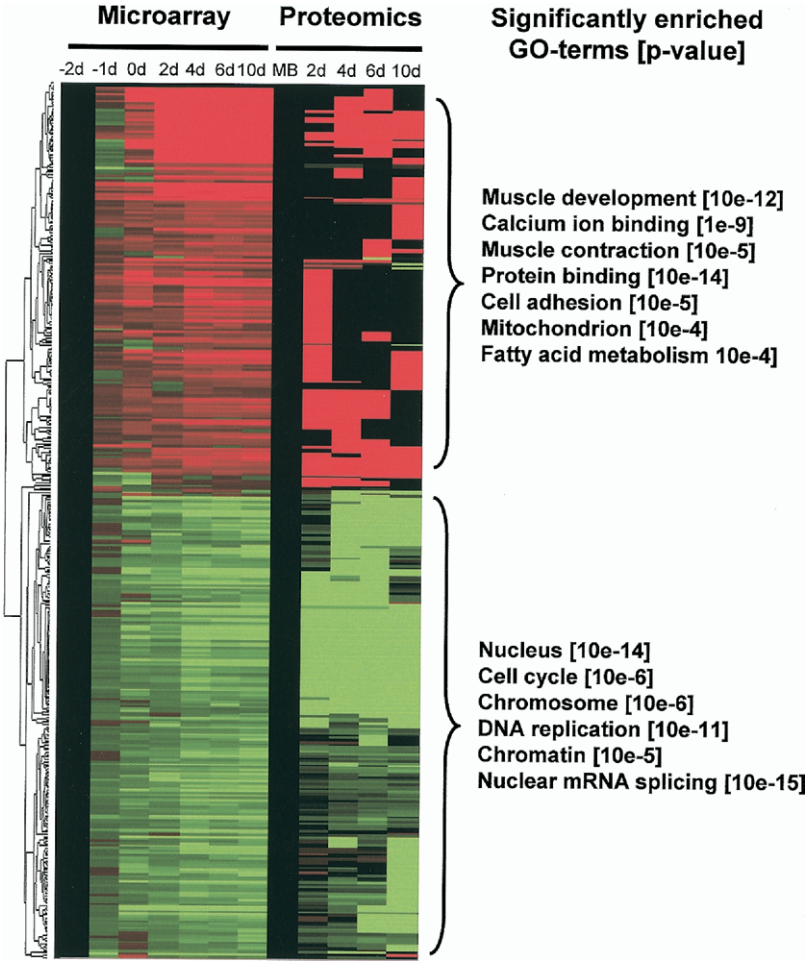

Figure 4. Comparison of microarray and proteomic informatic analysis of muscle cell development in culture. **Red** color denotes gene up-regulation, whereas **green** color indicates down-regulation. The result shows that there is a reasonable correspondence of genes seen in microarray and proteomics. However, there are also very unique species that are differentially regulated between gene expression and protein presence. This shows that the transcriptosomic and proteomic approaches are complementary and not duplicative. DNA = deoxyribonucleic acid; GO = Gene Ontology; mRNA = messenger ribonucleic acid. Reprinted with permission from Kislinger T, Gramolini A, Pan Y, et al. Proteome dynamics during C2C12 myoblast differentiation. Mol Cell Proteomics 2005;4:887–901.

subproteomes (Fig. 3). The serial analysis of the subproteome components in a complex protein mixture provides a much more balanced and detailed portrait of the sample being analyzed. This is particularly true for the myocardium, where large amounts of structural and mitochondrial proteins need to be separated first, to allow the in-depth analysis of individual subproteomes. However, the caveat is that clean separation of the subproteomes might not always be possible, and cross contamination might take place. Therefore, care must be taken to ensure that existing protocols or commercial products are performing equally well on cardiac myocytes as they do on other non filamentous cells or tissues.

Combinatorial genomic and proteomic analysis. To provide a biological context for the large-scale bioinformatic analysis of proteomic samples, we have been combining expression array and proteomic data from the same samples to maximize the confidence of the changes observed and to minimize the false positive rates. This significantly improved our ability to piece together complex network of pathways connecting the cell surface, genome, and subproteomes in both normal and diseased states. Our results indicate that there is a significant consonance of the transcriptosomic with proteomic changes (Fig. 4). The combination of approaches helps to prioritize the importance of various families of interacting pathways for further evaluation. However, this approach does not capture in detail the protein PTMs and processing that constantly alter the target function. This type of comparison also assumes equal breakdown and synthesis of proteins at equilibrium, which might or might not be true for individual proteins at any given time.

STRATEGIES FOR THE DISCOVERY OF BIOMARKERS IN PLASMA OR SERA

Accessible protein biomarkers of both normal and disease state are pivotal for a successful diagnostic/prognostic program, particularly if the disease state might be characterized by distinct fingerprints of the circulating proteins. Indeed, blood represents one of the most accessible sources for

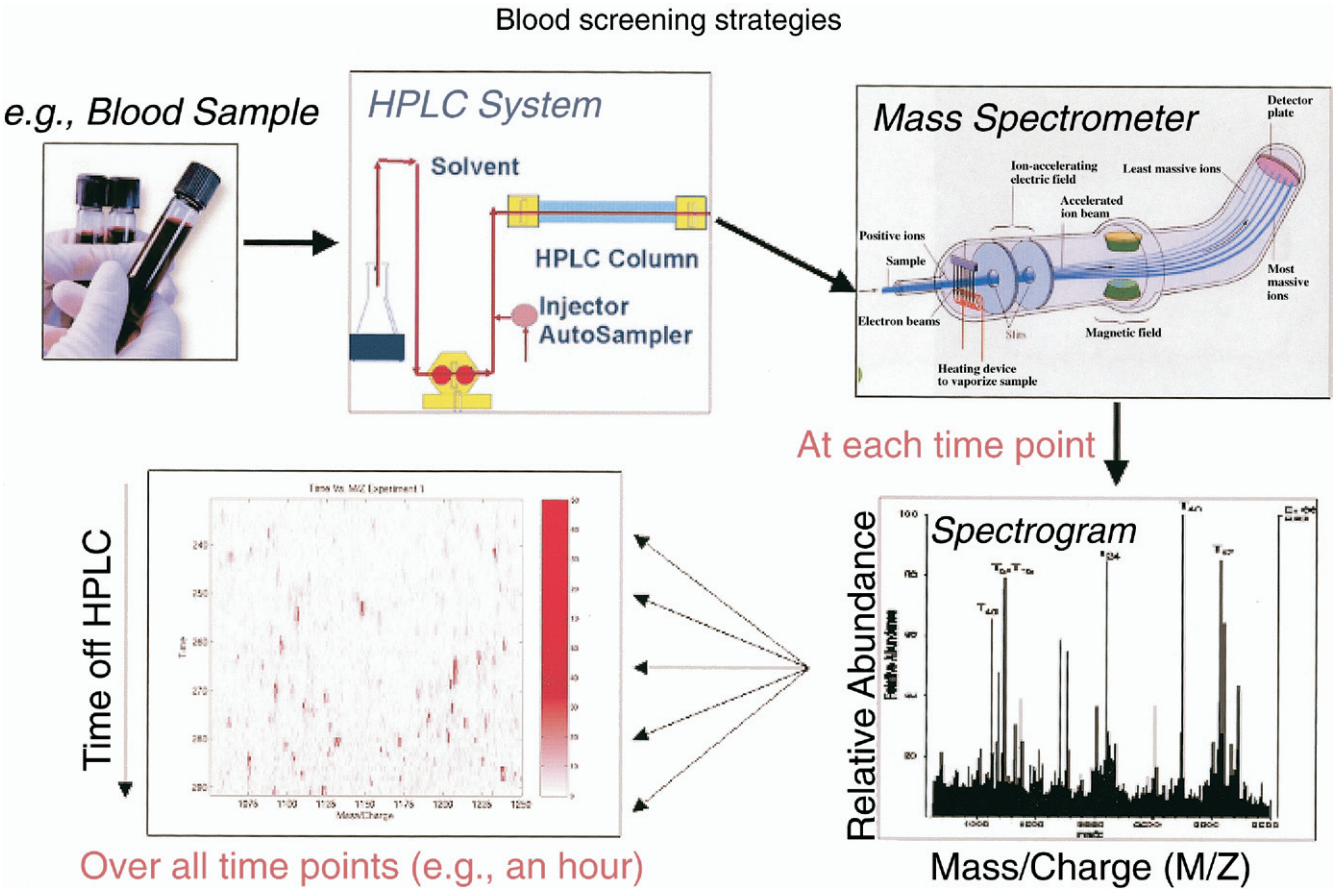

Figure 5. Screening or validation of protein peptide markers can again be conducted after sample separation with high-pressure liquid chromatography (HPLC), followed by mass spectrometer analysis. However, ability to remove overabundant species such as albumin or immunoglobulins will significantly facilitate the detection of smaller peptide species with higher sensitivity.

biomarkers and would have broad clinical significance. Fortunately the cardiovascular system is in constant intimate contact with the blood, making blood-based biomarker discovery a particularly worthwhile approach. However, other fluids, such as the urine, can also serve as potential sources in selected situations.

The choice of serum or plasma is often based on practical considerations of ease of sample collection and processing and whether anticoagulated containers are readily available. Additional considerations include whether the target proteins are involved or bound to the coagulation cascade, where plasma samples are needed, or whether there are interfering components in the plasma, where serum will be preferred. In practice, consideration of a uniform blood collection protocol to ensure consistency is more important than deciding whether it should be serum or plasma.

Although serum or plasma samples provide an excellent source of materials for proteomic analysis, the complexity of protein composition makes direct protein profiling from the blood extremely challenging. Plasma contains a dynamic range of protein concentrations from 55,000,000,000 pg/ml (e.g., albumin) to 1 to 5 pg/ml (e.g., IL-6), with a considerable number of proteins in between. A rough estimate of the number of proteins in human blood is over 10^6 different molecules representing products of about 25,000 to 30,000 genes (27). This extreme range in protein number and concentration demonstrates the challenge of identifying appropriate biomarkers in the blood. We discuss here the pros and cons of different approaches to blood-based biomarker discovery (Fig. 5).

Global approach with blood sample as the discovery tool. Although the direct analysis of the whole blood proteome to identify potential biomarkers is the most logical and intuitive starting point, the number of candidates will be restricted to abundant proteins, owing to limitations of current technologies. The combination of more sophisticated methods to deplete highly abundant proteins, with the use of capillary electrophoresis to detect low molecular weight proteins, might yield more proteomic biomarker candidates with time. The number of detectable proteins in plasma has risen dramatically from 40 proteins in 1975 (28) to over 3,000 in recent studies (29). Albumin depletion techniques have been used relatively successfully to improve the yield of lower molecular weight species in the plasma (30,31). However, the potential loss of albumin bound markers or proteins that are non-specifically bound to either the column matrix or the extracting antibodies are the trade-offs. Current challenges also include bioinformatic

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analysis to identify unique patterns of biomarkers that can accurately distinguish disease from normal, stage the disease, and provide prognostic information (32–34).

Targeted approach with discovery made in model systems first, then validation in blood. This approach to biomarker identification is based on the more traditional hypotheses-driven evaluation of specific candidates either on the basis of a biological rationale or analysis of candidates derived from other sources and then validated in blood samples. One way of approaching candidate biomarker discovery is the use of an appropriate repertoire of animal model systems. For example, in our program to discover novel biomarkers for heart failure, particularly those that might be specific for early, mid, and late stages of the disease, we use multiple genetic and acquired models of heart failure in murine and other animal species. The result of informatic analysis from these models is then integrated with tissue analysis from patients or explanted hearts with advanced forms of heart failure. This approach generates a large number of biomarker candidates in a biological, temporal, and clinically relevant context and permits the validation of these candidates in the serum or plasma of appropriately selected patient cohorts in replicates.

REMAINING CHALLENGES IN THE SEARCH FOR NOVEL PROTEOMIC BIOMARKERS

As the application of proteomics evolves in cardiovascular medicine, many challenges remain. For example, what are the advantages and trade-offs of the different technology platforms in evaluating the cardiovascular proteome? Is a combination approach better than any one platform alone? What should be a common protocol for blood sample handling and processing? What is the influence of standard cardiovascular drugs on the blood proteome? How do we integrate current protein data (35) with biomarker data from different investigators?

To coordinate communication amongst investigators interested in proteomics and to answer some of these questions in general for the entire proteomic community, HUPO was formed in 2001 with the objective of coordinating the global proteomic discoveries after the completion of the Human Genome Project (35,36). A cardiovascular initiative to help in answering these questions specifically for the cardiovascular proteome has also been established (37). This will help to coordinate the efforts of investigators in the field to solve these problems as a community and to develop common standards for the purpose of data exchange, pooling, and discovery.

CONCLUSIONS

As we incorporate the molecular and genomic advances into cardiovascular medicine in the coming era of more "personalized medicine," the rapid advances of proteomic technology opens a new window on the diseases that we are trying to treat and prevent. Proteins are the ultimate biological determinants of cardiovascular function and disease phenotype. Proteomics will not only help to unravel some of the remaining mysteries of biology of cardiovascular disease but permit the development of novel diagnostic and prognostic biomarkers useful to better care for our patients.

However, for clinicians and clinical investigators, it is very important to take advantage of the strengths of proteomics in the context of good study design, considerations of statistical power, and appropriate blinding and validation. Ultimately it is the robust study result in a clinically meaningful context that leads to clinical advances. Clinical phenotyping and accurate tracking of outcomes are still the cornerstones of good translational medicine.

It is also important to integrate the proteomic biomarker information with that available from genetic biomarkers, such as haplotype polymorphisms that determine disease susceptibility, to provide an integrated risk stratification. Proteomic biomarkers need to also be coordinated with traditional biochemical markers and clinical and image-based phenotype information, as an integrated database to make clinical decisions. No diagnostic tool, however accurate and powerful, can supplant the importance of a good clinical history and traditional phenotype information to establish the pre-test likelihood for any test. However, the development and validation of clinically relevant proteomic biomarker tools will likely provide the important information on early diagnosis, prognosis, and treatment efficacy on cardiovascular disease beyond what we can provide today and pave the way for personalized medicine of tomorrow.

Reprint requests and correspondence: Dr. Peter Liu, Heart & Stroke/Richard Lewar Centre of Excellence and Institute of Circulatory and Respiratory Health, Canadian Institute of Health Research, NCSB 11-1266, Toronto General Hospital, 200 Elizabeth Street, Toronto, Ontario M5G 2C4, Canada. E-mail: peter.liu@utoronto.ca.

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